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APPLICATION FOR UNITED STATES LETTERS PATENT

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TITLE: Methods for Measuring  
Bacteria, Bacteria Measuring  
Apparatuses, and Storage  
Media for Storing Computer-  
Executable Programs for  
Analyzing Bacteria

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METHODS FOR MEASURING BACTERIA, BACTERIA MEASURING  
APPARATUSES, AND STORAGE MEDIA FOR STORING COMPUTER-  
EXECUTABLE PROGRAMS FOR ANALYZING BACTERIA

5 FIELD OF THE INVENTION

The present invention relates to apparatuses, methods,  
and programs for automatically detecting bacteria in a  
sample and, more particularly, to apparatuses, methods, and  
programs for automatically detecting a bacillus or coccus  
10 bacterium in a sample.

BACKGROUND

Detection of bacteria contained in a sample and  
determination of the type of bacteria are often performed in  
15 clinical examinations and food sanitation examinations. The  
types of bacteria are typically classified by Gram  
stainability of the bacteria (e.g., Gram positive or Gram  
negative), and by shape (e.g., bacillus or coccus). Gram-  
negative bacillus and Gram-positive coccus frequently  
20 produce adverse effects on the human body.

The agar culture method is the most common method for  
classifying bacteria. This method involves culturing a  
sample in an agar medium for a predetermined time, either  
staining or not staining colonies formed in the culture, and  
25 having an observer classify the bacteria using a microscope.  
However, the agar culture method is a difficult process  
inasmuch as it is essentially a manual method. Furthermore,  
considerable time must elapse before the type of bacterium  
can be determined since culturing is required.

30 In recent years, methods have been tried which  
automatically measure bacteria using a particle analyzer,  
such as a flow cytometer or the like. This technology  
involves the following aspects: (1) a method and apparatus

for measuring microorganisms, which respectively measure pre-culture and post-culture samples to prevent measurement errors due to impurities in the sample by determining the difference between the two measurement results (e.g., see: United States Patent No. 6,165,740); and (2) a method for counting bacteria in samples containing impurities, which separates the bacteria from the impurities, counts the bacteria by adding a cationic surfactant to a sample containing bacteria so as to increase the colorant transmission of the bacteria. and stains the bacteria through the action of the colorant (e.g., see: European Patent Publication No. 1136563 A2).

Measurement can be accomplished in a relatively short time when the method automatically measures the bacteria by a particle measuring apparatus such as a flow cytometer or the like. However, among such methods, no technique has yet been proposed for differentiating bacillus and coccus with greater accuracy.

## SUMMARY

The scope of the present invention is defined solely by the appended claims, and is not affected to any degree by the statements within this summary.

A method for measuring bacteria embodying features of the present invention includes (a) fluorescently staining bacteria in a sample; (b) detecting size information from the bacteria in the sample, and fluorescence information expressing intensity of fluorescent light emitted by the bacteria; (c) creating a scattergram representing a distribution of the bacteria based on the size information and the fluorescence information detected; (d) analyzing the distribution of the bacteria in the scattergram; and (e) determining whether the bacteria in the sample is bacillus

or coccus based on a result of the analyzing.

A bacteria measuring apparatus embodying features of the present invention includes: (a) a sampling device for sampling a sample containing fluorescently stained bacteria; (b) a first detector for detecting size information from each bacterium in the sample; (c) a second detector for detecting fluorescence information expressing intensity of fluorescent light emitted from each bacterium in the sample; and (d) a control unit configured for creating a scattergram of the bacteria using the size information and the fluorescence information as parameters, for analyzing distribution of the bacteria in the scattergram, and for determining whether the bacteria in the sample is bacillus or coccus based on an analysis result.

A computer-executable program for analyzing bacteria embodying features of the present invention includes: (a) obtaining size information of bacteria and fluorescence information expressing intensity of fluorescent light emitted by the bacteria; (b) creating a scattergram representing a distribution of the bacteria based on the size information and the fluorescence information; (c) analyzing the distribution of the bacteria in the scattergram; and (d) determining whether the bacteria is bacillus or coccus based on a result of the analyzing.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a perspective view of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 2 is a perspective view of the sample preparation unit of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 3 is an illustration of the optical system and

flow system of the detection unit of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 4 is a block diagram of the analysis control unit of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 5 is a flow chart showing the overall control of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 6 is a flow chart showing the operation of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 7 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 8 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 9 illustrates the unit vector used in the analysis process performed by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 10 shows an example of a screen displayed by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 11 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 12 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 13 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 14 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

5 Fig. 15 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 16 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

10 Fig. 17 shows an example of a scattergram prepared by an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 18 is a flow chart of the operation of an automated bacteria measuring apparatus embodying features of the present invention.

Fig. 19 shows an example of a screen displayed by an automated bacteria measuring apparatus embodying features of the present invention.

## 20 DETAILED DESCRIPTION

The present invention provides techniques, apparatuses, and computer readable programs for rapidly and accurately determining whether the type of bacteria in a sample is bacillus or coccus.

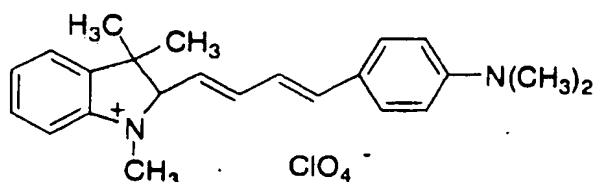
25 The methods for measuring bacteria in accordance with the present invention include (a) preparing a sample by fluorescently staining the bacteria in a specimen; (b) detecting size information of the bacteria and fluorescence information expressing the intensity of fluorescent light  
30 emitted by the bacteria of each type in a prepared sample; (c) creating a scattergram using the detected size information and fluorescence information as parameters; (d) analyzing the distribution of the bacteria in the

scattergram; and (e) determining whether the type of bacteria in the sample is bacillus or coccus based on the analysis result.

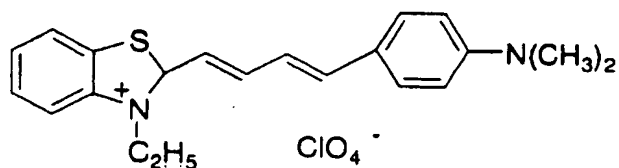
A fluorescent dye for bonding to components of the bacteria and emitting fluorescent light is used in the bacteria fluorescent stain. For example, bacteria in a specimen may be uniquely stained using a nucleic acid staining dye which uniquely bonds with intracellular DNA and RNA of the bacteria. By way of example, polymethene dyes having structures (1) through (11) below may be used:

(1) Thizole orange

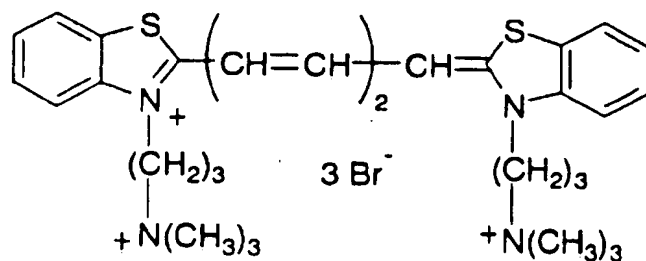
(2)



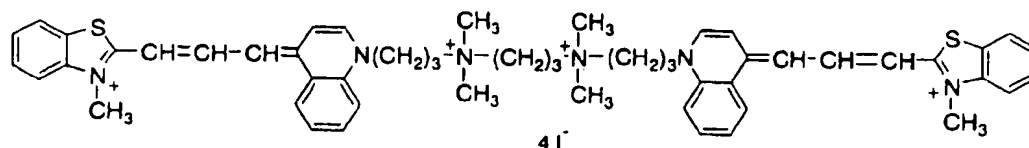
(3)



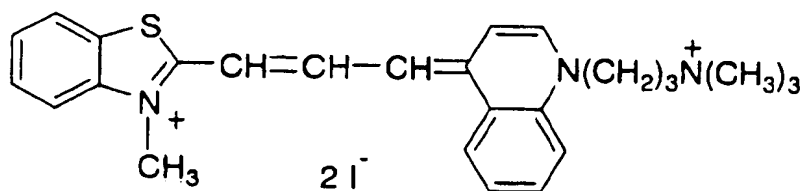
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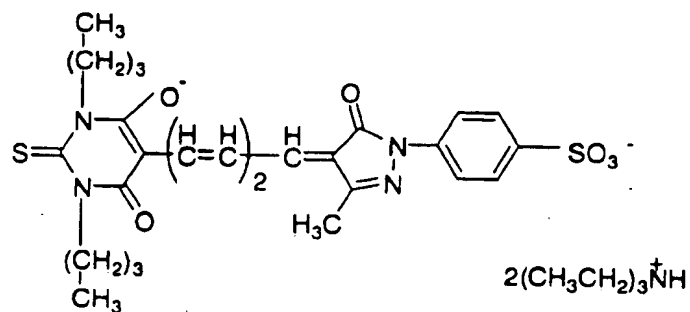
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(6)

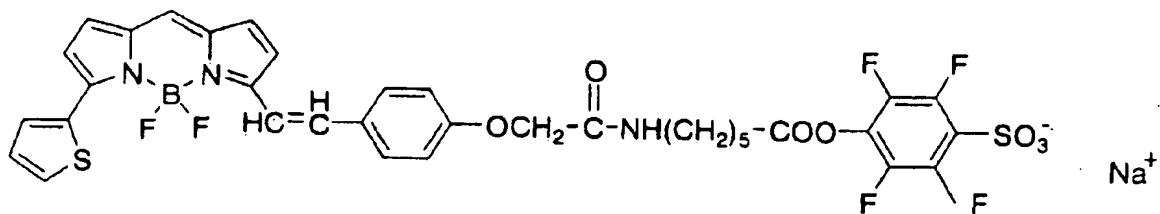


(7)

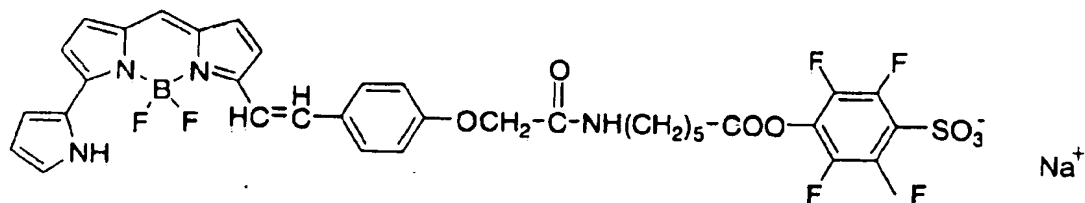


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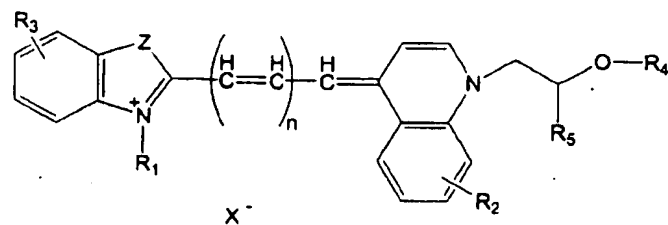
(8)



(9)



(10) Compounds having the general formula:



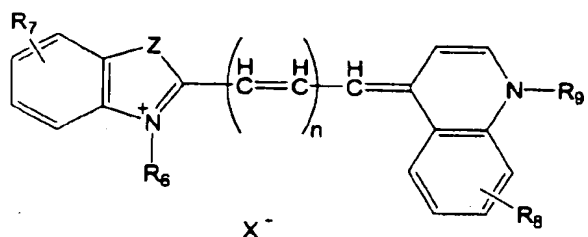
10

wherein,  $\text{R}_1$  represents a hydrogen atom or an alkyl group



having 1 to 3 carbon atoms; R<sub>2</sub> and R<sub>3</sub> represent hydrogen atoms, alkyl groups having 1 to 3 carbon atoms, or alkoxy groups having 1 to 3 carbon atoms; R<sub>4</sub> represents a hydrogen atom, acyl group, or alkyl group having 1 to 3 carbon atoms; R<sub>5</sub> represents a hydrogen atom or alkyl group having 1 to 3 carbons atoms which may optionally be substituted; Z represents a sulfur atom, oxygen atom or carbon atom substituted by two alkyl groups having 1 to 3 carbon atoms; n represents 1 or 2; and X represents an anion.

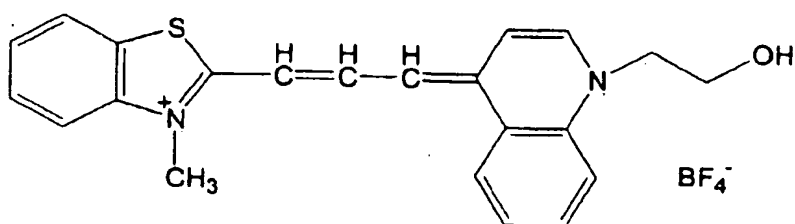
(11) Compounds having the general formula:



wherein, R<sub>6</sub> represents a hydrogen atom or alkyl group having 1 to 18 carbon atoms; R<sub>7</sub> and R<sub>8</sub> represent hydrogen atoms, alkyl groups having 1 to 3 carbon atoms, or alkoxy groups having 1 to 3 carbon atoms; R<sub>9</sub> represents a hydrogen atom, acyl group, or alkyl group having 1 to 18 carbon atoms; Z represents a sulfur atom, oxygen atom or carbon atom substituted by two alkyl groups having 1 to 3 carbon atoms; n represents 0, 1, or 2; and X represents an anion.

Among these dyes, (1) is commercially available, (2) and (3) are available from Japan Photosensitive Dye Research Center, and (5) through (9) are available from Molecular Probes, Inc. Dye (10) may be manufactured by the method described in United States Patent No. 5,821,127. Dye (11) may be manufactured by the method described in United States Patent No. 6,004,816.

Among the dyes represented by the general formula (10), the dye shown below is particularly desirable at present:



Bacteria having a specific level of fluorescent intensity may be distinguished from impurities which substantially lack fluorescent intensity if fluorescent information (fluorescent intensity) is detected from each particle in a sample subjected to a fluorescent staining process using the above-mentioned fluorescent dyes.

Electrical resistance methods, which use a detector having a member comprising a pore for passing through bacteria and first and second electrodes, detect the size of particles from the change in electrical resistance of a sample over time when the prepared sample passes through the pore. These methods may be used for detecting particle size information. Methods which detect scattered light pulse width or scattered light intensity emitted by particles using flow cytometry may also be used for detecting particle size information. In these methods, particle size information includes information reflecting particle diameter, particle width in a direction perpendicular to the particle diameter, particle volume or the like. The value of the change in electrical resistance detected by the electrical resistance method, and the pulse width and intensity of the scattered light signal detected by the flow cytometry method may be used as the size information.

Methods for detecting the intensity of fluorescent light emitted by fluorescently stained particles via flow cytometry may be used as the method for detecting fluorescence information.

Flow cytometry is a method in which laser light is used to irradiate a flowing sample fluid which contains target particles such as bacteria and cells. Optical information concerning scattered light and fluorescent light generated when the particles pass through the laser irradiation area is detected. Subsequently, the particles are analyzed based on the detected optical information. The various optical information is detected as pulse-like electrical signals by photoelectric conversion elements, such as photodiodes, photomultiplier tubes, and the like. Using these signals, signal intensity may be obtained based on the pulse peak height, and the light emission time may be obtained based on the pulse width. In general, the forward scattered light signal reflects the size of the particle, and the fluorescent light signal reflects the degree of staining of the particle which was fluorescently stained beforehand.

A scattergram is composed of dots corresponding to individual particles based on a plurality of particle information detected from each particle on a coordinate space having as its axes a plurality of types of information (e.g., size information expressing the size of the particle, and fluorescent light information), which reflect the characteristics of the particle. When the particle properties differ, differences occur in the distribution of dots on the scattergram. The present inventors have discovered that when bacillus-containing samples and coccus-containing samples were compared, the size information of both are substantially identical but the obtained fluorescent light information tends to be greater for the bacillus than for the coccus, which results in differences in the dot distribution on the scattergram. Thus, in accordance with the present invention, a determination as to whether bacteria in a sample is bacillus or coccus is based

on the differences in this distribution.

Differences in the distribution of dots in a bacillus specimen and coccus specimen are expressed in the slopes of the distributions. The slopes of the distributions are described below. Fig. 7 shows an example of a scattergram obtained from a specimen containing bacillus, and Fig. 8 shows an example of a scattergram obtained from a specimen containing coccus, wherein the fluorescent intensity (FL) is plotted on the X axis, and the forward scattered light intensity (Fsc) is plotted on the Y axis. Region BCT in the drawings is regarded as the region in which dots corresponding to bacteria appear. The population of dots corresponding to bacteria in the scattergrams is distributed so as to extend in a fixed direction (from lower left to upper right). In the coordinate space of the scattergrams, the slope having this "fixed direction" is the slope of the distribution. When Figs. 7 and 8 are compared, the slope relative to the X axis in the direction in which the population extends to the upper right (i.e., slope of the distribution) is larger in the coccus-containing specimen (Fig. 8) than in the bacillus-containing specimen (Fig. 7). From this fact, in accordance with the present invention, a determination as to whether bacteria in a specimen is bacillus or coccus is based on the slope of the distribution. The slope of the distribution is determined in the direction of maximum variance of the dots representing the bacteria, and the slope of the distribution may be determined by determining the slope in the maximum variance direction. A slope of approximate expression calculated from the dots representing the bacteria may also be used as the slope of the distribution.

An embodiment of the automated bacteria measuring apparatus of the present invention is described below. In

Fig. 1, an external view of an automated bacteria measuring apparatus 1 is indicated by the solid lines, and the internal structure is briefly indicated by the dashed lines. The foremost surface of the apparatus is provided with a liquid crystal touch panel 11 for inputting various types of settings and displaying measurement results, specimen cover 12, reagent cover 13, and start switch 14. When the specimen cover 12 is opened, a specimen may be set in the specimen holding unit provided within the apparatus. When the reagent cover 13 is opened, reagent may be set in the reagent holding unit provided within the apparatus. The specimen holding unit and the reagent holding unit are further described below.

In the internal structure of the apparatus indicated by the dashed lines, the top part accommodates an analysis control unit 400, which includes a microcomputer and various types of circuits, and the like. The bottom part nearest the front side accommodates a sample preparation unit 200 for preparing sample fluids. The bottom part on the back side accommodates a detection unit 300 for detecting signals from the bacteria in the sample fluid.

#### Sample Preparation Unit

Fig. 2 shows the sample preparation unit 200 of the automated bacteria measuring apparatus 1. The sample preparation unit 200 is provided with a specimen holding unit 201, reagent holding unit 202, incubator 204 as a reaction unit, and dispenser 205. A receptacle 201a for accepting a specimen is placed in the specimen holding unit 201. In the automated bacteria measuring apparatus 1, a dilution fluid and staining fluid are used as measurement reagents, and a receptacle 202a accommodating a dilution fluid and a receptacle 202b accommodating a staining fluid

are respectively set in the reagent holding unit 202. A receptacle 204a for reacting the specimen and the reagent is placed in the incubator 204. A dispenser 205 is movable vertically, forward and back, and side to side via a drive device, and suctions and discharges a set amount of fluid. The dispenser 205 respectively suctions predetermined amounts of specimen in the specimen receptacle 201a set in the specimen holding unit 201, and dilution fluid in the dilution receptacle 202a or staining fluid in the receptacle 202b set in the reagent holding unit 202. The dispenser 205 discharges the fluid to the receptacle 204a set in the incubator 204. The incubator 204 maintains a predetermined temperature, and reacts the specimen and reagent to prepare a sample. The prepared sample is sampled by the dispenser 205, and supplied to the sample receptacle 112. Details of the operation of the sample preparation unit 200 are further described below.

#### Detection Unit

Fig. 3 illustrates the optical system and flow system of the detection unit 300. A sheath flow cell 107 is used to flow the sample fluid supplied from the sample receptacle 112 of the sample preparation unit 200, and is connected to the sample receptacle 112. Furthermore, the sheath flow cell 107 is provided with a nozzle 113, which discharges sample fluid toward an orifice 111, a sheath fluid supply port 110, and drainage port 114. Near the sheath flow cell 107 are disposed a laser light source 117 for irradiating a laser beam on a sample fluid flowing in the sheath flow cell 107, and various types of optical components (condenser lens 118, beam stopper 119, collector lens 120, shield plate 130 having a pin-hole 121, dichroic mirror 122, and filter 123) for condensing the fluorescent light and forward scattered

light emitted from the particles in the sample fluid irradiated by the laser light. Furthermore, a photomultiplier tube 124 is provided as a detection device for detecting condensed fluorescent light, and a photodiode 125 is provided as a detection device for detecting forward scattered light.

A sheath fluid receptacle 109, which is pressurized by a positive pressure pump 147, is connected to the sheath fluid supply port 110 through a valve 105. The drainage port 114 is connected to a waste fluid chamber (not shown). The nozzle 113 is connected to the sample receptacle 112 through a valve 101, and to a negative pressure pump 148 through a flow path 139 and valve 102. A syringe pump 133 is connected on the valve 102 side of the flow path 139.

The detection unit 300 of the above-described structure detects forward scattered light signals and fluorescent light signals from particles of bacteria and the like included in a sample prepared in the sample preparation unit 200 as it flows through the sheath flow cell 107. The detected signals are transmitted to an analysis control unit 400. Detailed operation of the detection device 300 is described below.

#### Analysis Control Unit

Fig. 4 is a block diagram showing the structure of the analysis control unit 400. The analysis control unit 400 includes a computer incorporating a CPU, RAM, ROM, hard disk, and the like, and various types of circuits, and functions as an information processor 134 and a drive circuit 137. As shown in Fig. 4, the information processor 134 is provided with an analyzer 141, memory 145, and controller 146, and the analyzer 141 is provided with a scattergram generator 142, analysis unit 143, and

determining unit 144.

The memory 145 stores (a) analysis programs for analyzing, via the analyzer 141, the signals obtained from particles in the sample fluid by the detecting unit 300, and  
5 (b) control programs for controlling the operation of each part of the apparatus. The controller 146 controls the drive circuit 137 based on the control program. The drive circuit 137 drives the dispenser 205 shown in Fig. 2, syringe pump 133 shown in Fig. 3, valves 101, 102, 105, positive pressure  
10 pump 147, negative pressure pump 148, and laser light source 117 based on the control of the controller 146.

#### General Controls

Fig. 5 is a flow chart of the general control of the  
15 automated bacteria measuring apparatus 1 accomplished via the control program. After the receptacle 201a accommodating a specimen is placed in the specimen holding unit 201, the receptacle 202a accommodating a dilution fluid and receptacle 202b accommodating a staining fluid are placed in  
20 the reagent holding unit 202. The receptacle 204a for reacting the specimen and reagent (which is empty at this time) is placed in the incubator 204 of the sample preparation unit 200 (Fig. 2). When the start switch 14 is pressed, the control program starts, and step A (sample  
25 preparation unit control), step B (detection unit control), and step C (analyzer control) are sequentially executed. In this way, the sample preparation unit 200, detection unit 300, and analyzer 141 are controlled, and the series of operations of the automated bacteria measuring apparatus 1  
30 are automatically executed. Details of the operation of each part of the apparatus in steps A, B, and C are described below.



Step A (Control of the Sample Preparation Unit)

The operation of the sample preparation unit 200 by the sample preparation unit control is described below in reference to Fig. 2. First, the dispenser 205 measures a  
5 fixed quantity of the bacteria-containing specimen from the receptacle 201a of the sample holding unit 201, and dispenses 50  $\mu$ l into the receptacle 204a of the incubator 204. Next, the dispenser 205 measured a fixed quantity of dilution solution from the receptacle 202a of the reagent  
10 holding unit 202, and dispenses 340  $\mu$ l into the sample-containing receptacle 204a. The incubator 204 mixes the sample and the specimen and the dilution fluid for 10 seconds while maintaining a temperature of 42 °C. Then, the dispenser 205 measures a fixed quantity of staining fluid  
15 from the receptacle 202b of the reagent holding unit 202, and dispenses 10  $\mu$ l into the receptacle 204a. The incubator 204 shakes and mixes the fluids to induce reaction of the staining fluid while maintaining the receptacle 204a at 42 °C so as to prepare a sample. Approximately 400  $\mu$ l of the  
20 prepared sample is retrieved by the dispenser 205, and supplied to the sample receptacle 112. The sample in the sample receptacle 112 flows to the sheath flow cell 107 of the detection unit 300, as described below.

### Step B (Control of the Detection Unit)

The operation of the detection unit 300 by the detection unit control is described below in reference to Fig. 3. When the sample prepared by the sample preparation unit 200 is accommodated in the sample receptacle 112, the negative pressure pump 148 is actuated. Valves 101 and 102 are simultaneously opened, and the sample fills the flow path 139 between the valves 101 and 102 due to the negative pressure. Thereafter, the valves 101 and 102 are closed.

Next, the syringe pump 133 forces a fixed quantity of the sample in the flow path 139 into the nozzle 113, and the sample is discharged from the nozzle 113 into the sheath flow cell 107. Then, a sheath fluid is supplied from the sheath fluid container 109 to the sheath flow cell 107 by opening the valve 105 simultaneously with the discharge.

In this manner, the sample is surrounded by the sheath fluid, and the flow is constricted by the orifice 111. By constricting the flow of the sample, the particles contained in the sample fluid flow along in a linear alignment. When the sample passes through the orifice 111, the sheath fluid is discharged to the drain port 114.

A laser beam emitted from the laser light source 117 and constricted by the condenser lens 118 irradiates the sample flow 126 flowing through the orifice 111.

The laser light transmitted through the sheath flow cell 107 without irradiating the particles in the sample is blocked by the beam splitter 119. The forward scattered light and fluorescent light emitted from the particles irradiated by the laser beam are condensed by the collector lens 120, and pass through pin hole 121 of the light shield 130. This light then impinges the dichroic mirror 122.

The fluorescent light, which has a longer wavelength than the forward scattered light, is transmitted directly

through the dichroic mirror 122, detected by the photomultiplier tube 124 after the forward scattered light has been removed by the filter 123, and output as a fluorescent light signal 127 (a pulse-like electrical signal).

Furthermore, the forward scattered light is reflected by the dichroic mirror 122, received by the photodiode 125, and output as a forward scattered light signal 128 (a pulse-like electrical signal). Then, the fluorescent light signal 127 and the forward scattered light signal 128 are input to an information processor 134 shown in Fig. 4.

#### Step C (Control of the Analyzer)

When the fluorescent light signal 127 and forward scattered light signal 128 from the detection unit 300 are input to the information processor 134, the signals are analyzed by the analyzer 141. This forms step C (control of the analyzer) in the general control of the automated bacteria measuring apparatus 1. The operation of the analyzer 141 via the analyzer control is described below in reference to the flow chart of Fig. 6. Although the program representing this operation sequence is stored beforehand in the memory 145 together with other programs, this program also may be supplied from an external memory medium or communication network.

First, the fluorescent light signal 127 and the forward scattered light signal 128 detected by the detection unit 300 are input to the scattergram generator 142 of the analyzer 141 (S1).

The scattergram generator 142 calculates the forward scattered light intensity  $F_{sc}$  from the maximum peak value of the input forward scattered light signal 128 as particle size information. Similarly, the fluorescent light intensity

FL is calculated from the fluorescent light signal 127 as fluorescent light information. Then, a two-dimensional scattergram is created by plotting the obtained FL on the X axis, and the Fsc on the Y axis (S2).

5       The analysis unit 143 discriminates the dots corresponding to bacteria from the dots appearing on the generated two-dimensional scattergram, and counts the particles identified as bacteria (S3). Fig. 7 shows an example of a two-dimensional scattergram generated from a  
10       bacillus bacteria-containing specimen. As shown in Fig. 7, the region BCT in which the bacteria are focused is set beforehand by discriminating the bacteria from the other particles. In this manner, the particles appearing within the BCT region are regarded as bacteria, and the particles  
15       within the region BCT are counted as bacteria.

      The analysis unit 143 determines the variance of the dots of particles within the BCT region in the X-Y two-dimensional space, and determines the directional vector E in which there is maximum variance through the center of the  
20       variance. The determined directional vector E is shown in Fig. 7. The determined directional vector E is then converted to a unit vector (a vector having a length of 1), as shown in Fig. 9. The unit vector is broken down into a component in the X axis direction and a component in the Y  
25       axis direction, and the magnitude of the component in the Y axis direction is designated as P (S4). P is a value representing the degree of slope of the directional vector relative to the X axis.

      Fig. 8 shows an example of a two-dimensional  
30       scattergram generated from a coccus-containing specimen, and indicates the determined directional vector similar to Fig. 7. A comparison of Figs. 7 and 8 clearly shows that the degree of slope of the directional vector relative to the X

axis is greater in the coccus-containing specimen (Fig. 8) than in the bacillus-containing specimen (Fig. 7). Thus, the value of P is also greater in the coccus-containing specimen (Fig. 8) than in the bacillus-containing specimen (Fig. 7).

5 The determining unit 144 compares the value of P calculated by the analysis unit 143 to a predetermined value A (in this case,  $A=0.68$ ) (S5). When  $P \geq A$ , the bacteria included in the specimen is determined to be coccus (S6 and S7), whereas when  $P < A$ , the bacteria is determined to be bacillus (S6 and  
10 S8).

The result of the determination by the determining unit 144 is combined with the scattergram created in S2 and the bacteria count calculated in S3 and output to the liquid crystal touch panel 11 (S9). An example of the screen output  
15 to the liquid crystal touch panel 11 is shown in Fig. 10. The scattergram, bacteria determination result, and bacteria count are displayed. In the spaces indicating the determination result for the type of bacteria, a mark is displayed in the [Bacillus] category among the [Bacillus]  
20 and [Coccus] categories to indicate that the determination result is bacillus.

#### Specimen Measurement Result Examples

The measurement of a specimen using the automated  
25 bacteria measuring apparatus 1 described above and the bacteria type determination results are described below.

##### Specimens

Specimens (a) through (g) described below were used.

- 30 (a) Human urine containing E. aerogenes (bacillus).  
(b) Human urine containing E. coli (bacillus).  
(c) Human urine containing S. aureus (coccus).  
(d) Human urine containing S. epidermis (coccus).

(e) Heart infusion broth mixed with *E. coli* (bacillus)  
(bacteria count approximately  $6 \times 10^5$ /mL).

(f) Heart infusion broth mixed with *P. aeruginosa*  
(small type bacillus) (bacteria count approximately

5.  $6 \times 10^5$ /mL).

(g) Heart infusion broth mixed with *S. aureus* (coccus)  
(bacteria count approximately  $6 \times 10^5$ /mL).

#### Assay Reagents

10 The specimens were processed using the dilution fluids  
and staining fluids listed below as reagents in preparing  
assay samples.

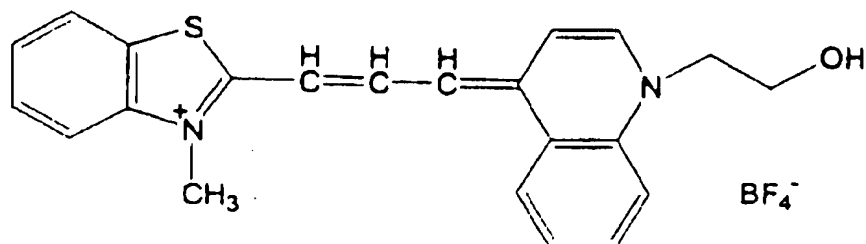
#### Dilution Fluids

Citric acid	100 mM
Sodium sulfate	90 mM
Amidosulfuric acid	100 mM
NaOH	enough to attain pH 2.5
Tetra decyltrimethylammonium bromide	1 g
Purified water	1 liter

15

#### Staining Fluids

Pigment having the structural formula below 40 mg



20

Ethylene glycol

1 liter

The two-dimensional scattergrams obtained from the measurement results of specimens (a), (b), (c), and (d) are shown in Figs. 11, 12, 13, and 14, respectively. In all cases, FL is plotted on the X axis (horizontal axis), and Fsc is plotted on the Y axis (vertical axis). The values of P calculated from the two-dimensional scattergrams in Figs. 11 through 14, and the bacteria type determination results based on the flow charts are shown in Table 1.

Table 1

Specimen	Bacteria type	P value	Determination result
(a)	<i>E. aerogenes</i> (bacillus)	0.27	Bacillus
(b)	<i>E. coli</i> (bacillus)	0.23	Bacillus
(c)	<i>S. aureus</i> (coccus)	0.83	Coccus
(d)	<i>S. epidermidis</i> (coccus)	0.77	Coccus

As shown in Table 1, the P values calculated from the bacillus-containing specimens are smaller than the P values calculated from the coccus-containing specimens. Furthermore, the bacteria type determination results match the actual bacteria types based on the result of the comparisons of the P value obtained from each specimen and the predetermined value A (in this case A=0.68).

The two-dimensional scattergrams obtained from the measurement results of specimens (e), (f), and (g) are shown in Figs. 15, 16, and 17, respectively. In all cases, FL is plotted on the X axis (horizontal axis), and Fsc is plotted on the Y axis (vertical axis). The values of P calculated from the two-dimensional scattergrams in Figs. 15 through 17, and the bacteria type determination results based on the flow charts are shown in Table 2.

Table 2

Specimen	Bacteria type	P value	Determination result
(e)	E. coli (bacillus)	0.35	Bacillus
(f)	E. aeruginosa (bacillus)	0.21	Bacillus
(g)	S. aureus (coccus)	0.74	Coccus

As shown in Table 2, the P values calculated from the  
5 bacillus-containing specimens are smaller than the P values  
calculated from the coccus-containing specimens.  
Furthermore, the bacteria type determination results match  
the actual bacteria types based on the result of the  
comparisons of the P value obtained from each specimen and  
10 the predetermined value A (in this case  $A=0.68$ ). When the  
large-type bacillus E. coli and the small-type bacillus P.  
aeruginosa are compared, the P. aeruginosa often appears at  
a position of lower value for Fsc in the two-dimensional  
scattergram, so that a difference arises in the state of  
15 distribution. However, both E. coli and P. aeruginosa may be  
accurately determined by the bacteria type determination  
based on the P value.

#### Determination Accuracy

20 Specimens of human urine which were bacteria typed by  
the plate agar culture method were measured using the  
automated bacteria measuring apparatus 1, and the bacteria  
typing determination accuracy is shown in Table 3.



Table 3

	Bacillus- containing specimens	Coccus- containing specimens	Total
Number of specimens	53	26	79
Number of matches	47	21	68
Accuracy(%)	88.7	80.1	86.1

As shown in Table 3, 47 matches (accuracy 88.7%) were obtained among the bacillus-containing specimens, and 21 matches (accuracy 80.1%) were obtained among the coccus-containing specimens. Furthermore, there were 68 matches (accuracy 86.1%) among a total of 79 bacillus-containing specimens and coccus-containing specimens. Accordingly, the bacteria measuring apparatus of the present embodiment has a high determination accuracy greater than 80%.

Fig. 18 shows a modification of the sequence of the flow chart shown in Fig. 6. This example only modifies the sequence when  $P < A$  in S6, and is otherwise identical to Fig. 6.

In the flow chart of Fig. 18, the determination unit 144 compares the parameter  $P$  and a predetermined value  $B$ , which is less than  $A$  (e.g.,  $B = 0.60$ ) when  $P < A$  (S6a). When  $P < B$  (S6b), the particles contained in the specimens are determined to be bacillus (S8), and when  $A > P \geq B$ , determination is deemed difficult (S6b and S6c).

Screens corresponding to these determination results are output to the liquid crystal touch panel 11 (S9). Furthermore, the scattergram created in S2, and the bacteria count calculated in S3 are combined and output to the liquid crystal touch panel 11. An example of the screen output on the liquid crystal touch panel 11 is shown in Fig. 19. The two-dimensional scattergram, bacteria typing determination result, and bacteria count are displayed. In the spaces

indicating the determination result for the type of bacteria, a mark is displayed in the [Determination Difficult] category among the [Bacillus], [Coccus], and [Determination Difficult] categories to indicate that the bacteria typing determination result for this specimen is difficult.

When  $A > P \geq B$  in S6b of the flow chart in Fig. 18, and determination is difficult, an indication that determination is difficult is output as shown in Fig. 19 without determining whether the bacteria type is bacillus or coccus. However, the presence of bacteria whether bacillus or coccus may be suggested, or the possibility of the presence of bacteria may be indicated without suggesting the type.

Furthermore, when outputting the bacteria typing determination result, the degree of reliability of the determination result may also be output. For example, when bacteria in a specimen is determined to be either bacillus or coccus, the degree of dissociation of the calculated P value and the predetermined value A may be calculated and output, as in the flow chart of Fig. 6. The larger the degree of dissociation, the greater the reliability of the determination result. When either [Bacillus], [Coccus], or [Determination Difficult] is determined as in the flow chart of Fig. 18, when coccus is determined ( $P > A$ ), the degree of dissociation between the P value and the predetermined value A is calculated. The larger the degree of dissociation, the higher the reliability of a result determining the bacteria is coccus. When bacillus is determined ( $P < B$ ), the degree of dissociation between the P value and the predetermined value B is calculated. The greater the degree of dissociation, the higher the reliability of the result determining the bacteria is bacillus.

Although the present invention has been described above

in terms of examples of presently preferred embodiments, the present invention is not limited to these examples. The present invention easily determines whether bacteria in a specimen is bacillus or coccus from the distribution of a scattergram created by bacteria size information and fluorescent light information. Since bacteria cultures are unnecessary in the present invention, it is possible to determine the bacteria type extremely quickly and efficiently.

The foregoing detailed description has been provided by way of explanation and illustration, and is not intended to limit the scope of the appended claims. Many variations in the presently preferred embodiments illustrated herein will be obvious to one of ordinary skill in the art, and remain within the scope of the appended claims and their equivalents.